UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

May 3, 2010

MEMORANDUM

Subject: Efficacy Review for Enviro San, EPA Reg. No. 1677-185; DP Barcode: D374121

From: Ibrahim Laniyan, Microbiologist
Product Science Branch
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Thru: Tajah Blackburn, Team Leader
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To: Demson Fuller / Marshall Swindell
Regulatory Management Branch I
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Applicant: Ecolab Inc.
370 N. Wabasha Street
St. Paul, MN 55102

Formulation from the Label:

Active Ingredient	% by wt.
Hydrogen Peroxide	11.2 %
Peroxyacetic Acid	15.2 %
Inert Ingredients:	73.6 %
Total	100.0 %

I. BACKGROUND

The product, Enviro San (EPA Reg. No. 1677-185), is an EPA-approved sterilant, sanitizer, sanitizing rinse, and antimicrobial rinse for use on hard, non-porous surfaces in commercial and food processing environments. The label claims that the product is effective as a sterilant in the presence of 500 ppm hard water. The applicant requested that EPA amend the registration to include use of the product, Enviro San, with the adjuvant ES-2000 at 50°C in commercial sterilization of food packaging materials and equipment. Studies were conducted at Ecolab Inc., Ecolab Research Center – Ecolab Schuman Campus, located at 655 Lone Oak Drive, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated December 21, 2009), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), a Certification with Respect to Label Integrity, two studies (MRID 479409-01 and -02), Statements of No Data Confidentiality Claims for both studies, and the proposed label.

Note: KX-8125, the adjuvant used during efficacy testing, was confirmed to be ES-2000, the new adjuvant identified on the proposed label.

II. USE DIRECTIONS

The product is designed for sterilizing surfaces such as non-porous manufacturing, filling, and packaging materials and equipment for food processing. The proposed label indicates that the product may be used on surfaces such as aluminum, glass, glazed porcelain, plastic (i.e., polyethylene, polypropylene), and stainless steel. [The proposed label includes directions for use on non-porous surfaces only.] Directions on the proposed label provided the following information regarding preparation and use of the product as a sterilant: Remove gross soil particles from surfaces. Thoroughly clean surfaces and follow with a potable water rinse. Prepare a use solution by adding 3.7 ounces (or 5.0 ounces) of the product and 0.01 ounces of the adjuvant (i.e., ES-2000) to 1 gallon of water. Apply the use solution by immersion, coarse spray, or circulation techniques. Surfaces should be exposed to the use solution for a period of not less than 35 seconds (or not less than 15 seconds when using 5.0 ounces of product and 0.01 ounces of adjuvant to 1 gallon of water) at 50°C. After thorough draining, rinse surfaces with disinfected or sterile water.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sterilizers: The AOAC Sporicidal Activity of Disinfectants Method is recommended for substantiating sterilizing claims. The following information applies to all products represented as sporicidal or sterilizing agents. Sixty carriers, representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops), must be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 product samples representing 3 different product lots, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) that is recommended for use in a specific device must be tested using the AOAC Sporicidal Activity of Disinfectants Method in that specific device and according to the directions for use of that specific device. Killing on all of the 720 carriers is required; no failures are permitted. Data to support sterilizing claims must be confirmed by tests conducted by a second, independent laboratory of the applicant's choice

(other than the laboratory that developed the original data). The following minimal confirmatory data must be developed on one sample of the product: Thirty carriers, representing each of the 2 types of surfaces (porcelain penicylinders and silk suture loops) against spores of both *Bacillus subtilis* and *Clostridium sporogenes* (a total of 120 carriers) by the AOAC Sporicidal Activity of Disinfectants Method. Killing on all of the 120 carriers is required; no failures are permitted.

Supplemental Claims: On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 479409-01 "Commercial Sterilant Efficacy of Enviro San at 3000 ppm Peroxyacetic Acid with KX-8125 Adjuvant (EPA REG. No. 1677-185)," by Laurinda Holen. Study conducted at Ecolab Inc. Study completion date – December 21, 2009. Study Identification Number 0900019.

This study was conducted against *Bacillus subtilis* (ATCC 19659), *Clostridium sporogenes* (ATCC 3584), and *Bacillus cereus* (ATCC 14579). Three lots (Lot Nos. J031891, J051291, and J070891) of the product, Enviro San, combined with three lots (Lot Nos. 4901160683, 4901092660, and 4900781255), of the adjuvant, KX-8125, were tested. The study referenced the AOAC Sporicidal Activity of Disinfectants Method as described in the AOAC Official Methods of Analysis, 18th Edition, 2006, modified for commercial sterilant testing. Each lot of product and each lot of adjuvant was at least 60 days old at the time of testing. Use solutions were prepared by adding ~22 g of the product and ~978 g of 500 ppm AOAC synthetic hard water (titrated at 480-520 ppm) to achieve 3000 ppm peroxyacetic acid and then dosing the solution with the adjuvant to achieve 0.254 Baker Units (U/g) catalase. [Use solutions were placed in a 50±2°C water bath for 45-60 minutes to allow the adjuvant and hydrogen peroxide present to degrade. Use solutions were then spiked with hydrogen peroxide to yield 500 ppm hydrogen peroxide in the use solutions. This preparation approach allowed testing to be conducted using the highest allowable concentration of hydrogen peroxide, a worst-case scenario as greater efficacy is achieved using a higher concentration of peroxyacetic acid and maintaining a lower concentration of hydrogen peroxide.] Cultures of the challenge microorganisms were prepared as followed: (1) *Bacillus subtilis* and *Bacillus cereus* cultures were grown in AOAC nutrient broth for 44-76 hours at 35±2°C, vortexed mixed, inoculated onto amended nutrient agar plates, and incubated for 8-14 days at 35±2°C; (2) *Bacillus subtilis* and *Bacillus cereus* cultures were harvested, subjected to centrifugation and re-suspension steps, and stored for no more than 90 days at 2-8°C; (3) the *Clostridium sporogenes* culture was grown in Brain Heart Infusion Broth anaerobically for 48±4 hours at 35±2°C, vortexed mixed, inoculated onto Brain Heart Infusion agar plates, and incubated for 6-12 days at 35±2°C; and (4) the *Clostridium sporogenes* culture was harvested, subjected to centrifugation and re-suspension steps, and stored for no more than 90 days at 2-8°C. The prepared suspensions were diluted as necessary with sterile Milli-Q water to achieve carrier counts between 1.0×10^5 and ~1×10^6 spores/carrier. Sixty (60) polished stainless steel penicillin cup carriers per product lot per test organism were inoculated with a suspension of test organism, at a ratio of 1 carrier per 1 mL of suspension. Carriers were allowed to soak in the suspension for 15-25 minutes at room temperature. The product was aspirated off and the carriers were placed on end in a

vertical position on sterile Petri dishes. The carriers were transferred to a vacuum desiccator containing $CaCl_2$ or equivalent desiccant. The vacuum was drawn to ≥69 cm Hg for ≥20 minutes and the carriers were dried under vacuum for ≥24 hours (i.e., 4 to 21 days). The carriers were transferred to individual tubes containing 10 mL of the use solution for 35 seconds at 50±2°C. Following exposure, each carrier was transferred to individual tubes of Fluid Thioglycollate with 0.5% sodium thiosulfate. Each carrier was then transferred to a secondary subculture tube of Fluid Thioglycollate with 0.5% sodium thiosulfate. Subculture tubes were incubated for 21 days at 35±2°C. Following incubation, the subculture tubes were visually examined for growth. Tubes demonstrating no growth after 21 days were heat-shocked for 20±2 minutes at 80±2°C and re-incubated for an additional 72±4 hours at 35±2°C. Controls included those for purity, sterility, viability (i.e., positive control), enumeration of the test systems, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided data for a number of failed studies. Carrier enumeration controls were invalid for *Bacillus subtilis* for September 11, 2009 and for *Clostridium sporogenes* for October 23, 2009, November 4, 2009, November 9, 2009, and November 10, 2009. Assay results were discarded prior to results being read. Testing was repeated. See Appendix B of the laboratory study.

2. MRID 479409-02 "Commercial Sterilant Efficacy of Enviro San at 4000 ppm Peroxyacetic Acid with KX-8125 Adjuvant (EPA REG. NO. 1677-185)," by Laurinda Holen. Study conducted at Ecolab Inc. Study completion date – December 21, 2009. Study Identification Number 0900018.

This study was conducted against *Bacillus subtilis* (ATCC 19659), *Clostridium sporogenes* (ATCC 3584), and *Bacillus cereus* (ATCC 14579). Three lots (Lot Nos. J031891, J051291, and J070891) of the product, Enviro San, combined with three lots (Lot Nos. 4901160683, 4901092660, and 4900781255), of the adjuvant, KX-8125, were tested. The study referenced the AOAC Sporicidal Activity of Disinfectants Method as described in the AOAC Official Methods of Analysis, 18th Edition, 2006, modified for commercial sterilant testing. Each lot of product and each lot of adjuvant was at least 60 days old at the time of testing. Use solutions were prepared by adding ~29 g of the product and ~970 g of 500 ppm AOAC synthetic hard water (titrated at 500-520 ppm) to achieve 4000 ppm peroxyacetic acid and then dosing the solution with the adjuvant to achieve 0.254 Baker Units (U/g) catalase. [Use solutions were placed in a 50±2°C water bath for 45-60 minutes to allow the adjuvant and hydrogen peroxide present to degrade. Use solutions were then spiked with hydrogen peroxide to yield 500 ppm hydrogen peroxide in the use solutions. This preparation approach allowed testing to be conducted using the highest allowable concentration of hydrogen peroxide, a worst-case scenario as greater efficacy is achieved using a higher concentration of peroxyacetic acid and maintaining a lower concentration of hydrogen peroxide.] Cultures of the challenge microorganisms were prepared as followed: (1) *Bacillus subtilis* and *Bacillus cereus* cultures were grown in AOAC nutrient broth for 44-76 hours at 35±2°C, vortexed mixed, inoculated onto amended nutrient agar plates, and incubated for 8-14 days at 35±2°C; (2) *Bacillus subtilis* and *Bacillus cereus* cultures were harvested, subjected to centrifugation and re-suspension steps, and stored for no more than 90 days at 2-8°C; (3) the *Clostridium sporogenes* culture was grown anaerobically in Brain Heart Infusion Broth for 48±4 hours at 35±2°C, vortexed mixed, inoculated onto Brain Heart Infusion agar plates, and incubated for 6-12 days at 35±2°C; and (4)

the *Clostridium sporogenes* culture was harvested, subjected to centrifugation and re-suspension steps, and stored for no more than 90 days at 2-8°C. The prepared suspensions were diluted as necessary with sterile Milli-Q water to achieve carrier counts between 1.0×10^5 and ~1×10^6 spores/carrier. Sixty (60) polished stainless steel penicillin cup carriers per product lot per test organism were inoculated with a suspension of test organism, at a ratio of 1 carrier per 1 mL of suspension. Carriers were allowed to soak in the suspension for 15-25 minutes at room temperature. The product was aspirated off and the carriers were placed on end in a vertical position on sterile Petri dishes. The carriers were transferred to a vacuum desiccator containing $CaCl_2$ or equivalent desiccant. The vacuum was drawn to ≥69 cm Hg for ≥20 minutes and the carriers were dried under vacuum for ≥24 hours (i.e., 6 to 13 days). The carriers were transferred to individual tubes containing 10 mL of the use solution for 15 seconds at 50±2°C. Following exposure, each carrier was transferred to individual tubes of Fluid Thioglycollate with 0.5% sodium thiosulfate. Each carrier was then transferred to a secondary subculture tube of Fluid Thioglycollate with 0.5% sodium thiosulfate. Subculture tubes were incubated for 21 days at 35±2°C. Following incubation, the subculture tubes were visually examined for growth. Tubes demonstrating no growth after 21 days were heat-shocked for 20±2 minutes at 80±2°C and re-incubated for an additional 72±4 hours at 35±2°C. Controls included those for purity, sterility, viability (i.e., positive control), enumeration of the test systems, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided data for a failed study set up on September 8, 2009. In the study, carrier enumeration controls were invalid for *Bacillus subtilis*. Control results were discarded prior to results being read. Testing was repeated. See Appendix B of the laboratory study.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Count (CFU/ carrier)
		Lot No. J031891 + 4901160683	Lot No. J051291 + 4901092660	Lot No. J070891 + 4900781255	
Pre-Heat Shock Results					
479409-01	*Bacillus subtilis* Test Date: 10/02/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60[1]	1° = 0/60 2° = 0/60	2.7×10^5
	Bacillus subtilis Test Date: 10/26/2009	--- ---	1° = 0/60 2° = 0/60	--- ---	7.5×10^5
	Clostridium sporogenes Test Date: 09/29/2009	1° = 0/60 2° = 0/60[1]	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	5.4×10^5
	Clostridium sporogenes Test Date: 11/16/2009	1° = 0/60 2° = 0/60	--- ---	--- ---	2.2×10^5
	Bacillus cereus Test Date: 09/25/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 2/60[2]	7.2×10^5
	Bacillus cereus Test Date: 10/09/2009	--- ---	--- ---	1° = 0/60 2° = 0/60	2.4×10^5

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Count (CFU/ carrier)
		Lot No. J031891 + 4901160683	Lot No. J051291 + 4901092660	Lot No. J070891 + 4900781255	
	Bacillus cereus Test Date: 10/16/2009	--- ---	--- ---	1° = 0/60 2° = 0/60	8.4×10^5
479409-02	*Bacillus subtilis* Test Date: 09/24/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60[1]	3.3×10^5
	Bacillus subtilis Test Date: 10/14/2009	--- ---	--- ---	1° = 0/60 2° = 0/60	1.4×10^5
	Clostridium sporogenes Test Date: 09/21/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	2.6×10^5
	Bacillus cereus Test Date: 09/18/2009	1° = 0/60[1] 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1.0×10^6
	Bacillus cereus Test Date: 10/19/2009	1° = 0/60 2° = 0/60	--- ---	--- ---	8.4×10^5
Post-Heat Shock Results					
479409-01	*Bacillus subtilis* Test Date: 10/02/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/59[3]	1° = 0/60 2° = 0/60	2.7×10^5
	Bacillus subtilis Test Date: 10/26/2009	--- ---	1° = 0/60 2° = 0/60	--- ---	7.5×10^5
	Clostridium sporogenes Test Date: 09/29/2009	1° = 0/60 2° = 0/59[3]	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	5.4×10^5
	Clostridium sporogenes Test Date: 11/16/2009	1° = 0/60 2° = 0/60	--- ---	--- ---	2.2×10^5
	Bacillus cereus Test Date: 09/25/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/58[4]	7.2×10^5
	Bacillus cereus Test Date: 10/09/2009	--- ---	--- ---	1° = 0/60 2° = 0/60	2.4×10^5
	Bacillus cereus Test Date: 10/16/2009	--- ---	--- ---	1° = 0/60 2° = 0/60	8.4×10^5
479409-02	*Bacillus subtilis* Test Date: 09/24/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/59[3]	3.3×10^5
	Bacillus subtilis Test Date: 10/14/2009	--- ---	--- ---	1° = 0/60 2° = 0/60	1.4×10^5
	Clostridium sporogenes Test Date: 09/21/2009	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	2.6×10^5
	Bacillus cereus Test Date: 09/18/2009	1° = 0/59[3] 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1.0×10^6
	Bacillus cereus Test Date: 10/19/2009	1° = 0/60 2° = 0/60	--- ---	--- ---	8.4×10^5

[1]One tube was positive for growth. The growth was shown to be a Gram positive cocci and the test system is a Gram positive rod. Growth, therefore, was confirmed not to be the test system. Efficacy testing was repeated for the product lot to verify efficacy against the test system.

[2]Efficacy testing was repeated for the product lot to verify efficacy against the test system. The laboratory noted that the use solution was prepared to 2999 ppm peroxyacetic acid (rather than 3000 ppm peroxyacetic acid).

[3]One positive tube confirmed not to be the test system was not heat shocked.

[4]Two positive tubes confirmed to be the test system were not heat shocked.

VI. CONCLUSIONS

1. The efficacy data provided for the additional sterilant use of the product with adjuvant (MRID 479409-01 and 479409-02) **support** use of the product, Enviro San, as a sterilant against the following microorganisms on hard, non-porous surfaces in the presence of 500 ppm hard water for the listed conditions:

Bacillus subtilis	35 seconds; 50°C; product plus adjuvant, 3000 ppm
Clostridium sporogenes	35 seconds; 50°C; product plus adjuvant, 3000 ppm
Bacillus cereus	35 seconds; 50°C; product plus adjuvant; 3000 ppm
Bacillus subtilis	15 seconds; 50°C; product plus adjuvant; 4000 ppm
Clostridium sporogenes	15 seconds; 50°C; product plus adjuvant; 4000 ppm
Bacillus cereus	15 seconds; 50°C; product plus adjuvant; 4000 ppm

Initial/basic efficacy data provided for the additional sterilant use of the product demonstrated complete killing in the subcultures of the required number of carriers (i.e., 360 hard, non-porous carriers) tested against the required number of product lots (i.e., 3). Neutralization confirmation testing showed positive growth of the microorganisms. The viability controls were positive for growth. Purity controls were reported as pure. The sterility controls did not show growth. Test spores showed resistance to acid for at least 2 minutes.

Note: The product was not tested using silk suture loops. Note that the proposed label includes directions for use on non-porous surfaces only.

VII. LABEL

1. The efficacy data provided support the additional sterilant use of the product with adjuvant ES-2000 for the following conditions:

(1) **35 seconds contact time** for use of the product, Enviro San at 3.7 oz. per gallon of water (4838 ppm peroxyacetic acid but no less than 3000ppm), with adjuvant ES-2000 (0.01 oz per gallon of water), at 50°C.

(2) **15 seconds contact time** for use of the product use of the product, Enviro San at 5.0 oz. per gallon of water (6597ppm peroxyacetic acid but no less than 4000ppm), with adjuvant ES-2000 (0.01 oz per gallon of water), at 50°C.

2. The applicant must make the following change to improve the proposed label:

- Under the "Container Disposal" section of the product label, identify disposal options for the ≤55-gallon rigid containers. The label states "offer for recycling, if available" and does not provide another option.